Titr of saturate 5' end with
Taq (see P.29)

ag N

Taq assay mix (P557)
except no activated DNA

	A		(40 Rxns)
0.5M Taps pH8.3	100		✓
1M MgCl2	4		✓
3M KCl	33.3 μl		✓
dATGC-TP 10mM each	40		✓
α32P dCTP	5.1 4.2 μl		✓
H2O	1.219 ml		✓
	1.4 ml		

(1) 385 → (2) 385 (use 3) μl/50 μl Rxn)

	(1)	(2)	
(same as P17) 3·mp18 500 pmol nt/μl 0.165 μg/μl)	13.3 μl	66.7 μl	← 11 Rxns (40 μl/50 μl)
H2O	96.7	433	
	495	495	

① = 0.2 μg DNA/50 μl
② = 1 μg DNA/50 μl
(= 5 nmol nt/50 μl)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

(1) 45 μl → (tubes 1–10)
(2) 45 μl → (tubes 11–20)

EKBTI			total units/50
.8	5	5	0.4
.16	5	5	0.8
.125	5	5	1.5
.25	5	5	3.125
	5	5	6.25
	5	5	12.5
	5	5	25
	5	5	50
	5	5	100
4 μl	5	5	200

50 μl

2 min at 74°C → add 10 μl EDTA
(spot 40 μl on GFC

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units/50µl			pmol
0.4	1	133.00	2.6
0.8	2	248.00	4.9
1.6	3	264.00	5.2
3	4	470.00	9.2
6	5	633.00	12.5
12	6	886.00	17
25	7	991.00	19.5
50	8	995.00	19.6
100	9	999.00	19.7
200	10	883.00	17.4
.4	11	2146.00	42
.8	12	3847.00	76
1.6	13	6695.00	132
3	14	12077.00	238
6	15	17179.00	339
12	16	17333.00	342
25	17	22279.00	440
50	18	22941.00	452
100	19	23863.00	471
200	20	24510.00	477
	21	92.00	
5λ of (2)	22	304197.00	76 CPM/pmol

(rows 1–10: 0.2 µg DNA; rows 11–20: 1.0 µg DNA)

need time course at high and low [Taq] to see if lag plays a role. ~~In~~ In a PCR with 15-30 min elongation time, effect of lag would be minimized

Results per Taq spec't = 100,000 u/mg

$\frac{25 \text{ units}}{50 \mu l}$ = 50 nM Taq

Both plots (0.2 and 1.0 µg DNA) saturate c 50 nM Taq suggesting an equilibrium effect of pol DNA binding rather than titration of pol att'l pol/length ~~[illegible]~~

1 µg DNA, saturation at 10 u/0.42 pmol circles ~~[illegible]~~

~~for 200,000 u/mg Taq~~

1 unit Taq = .053 pmol molecules ~ 1.1 Taq/circle

1 µg mp19 ⟹ $\frac{1 \times 10^{-6} \text{ g}}{(330 \text{ g mole wt})(7250 \text{ bp})}$ = 0.42 pmol circle

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